

THE EFFECT OF PARROT FISH THYROID EXTRACT ON THE RESPIRATORY METABOLISM OF THE WHITE RAT^{1, 2}

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The discovery of a discrete, encapsulated thyroid gland in the parrot fish (Matthews, 1948) made possible studies on the teleost thyroid which had hitherto been undertaken only with the greatest difficulty. Extracts of the fish thyroid became obtainable for the first time with relative ease. Such extracts, when injected into white grunts, significantly elevated the oxygen consumption in a certain proportion of the fish so treated (Smith and Matthews, 1948). The thyroids of parrot fish were found to concentrate iodine in a manner reminiscent of similar behavior in the glands of mammals (Matthews and Smith, 1948). There was thus established a certain parallelism between the action of teleost and mammalian thyroids and it was thought worthwhile to investigate the effect of parrot fish thyroid extract upon the respiratory metabolism of the white rat.

METHODS

The extracts used in these studies were prepared from thyroid glands collected in Bermuda and Bimini. With rare exceptions, the glands were taken from *Pseudo-scarus guacamaia*. As previously described (Smith and Matthews, 1950), the thyroid glands were removed immediately after the death of the fish and were placed in either acetone or absolute alcohol. Dehydration and defatting continued for at least two days with alternate changes of acetone or absolute alcohol. At the end of this period the glands were dried in partial vacuum and stored, either powdered or whole, in a desiccator until prepared for injection. The final average weight of the glandular material was 17 per cent of the wet weight of the gland when removed from the fish.

Extracts were prepared by dissolving the desired amount of dried glandular tissue in 2 cc. of 2N NaOH and bringing the resultant solution to neutrality with concentrated HCl. This procedure usually precipitated most of the dissolved material, but the resultant suspension was fine enough to be drawn through a 20 gauge needle and injected into the test animal. As a rule approximately 100 mgm. of dried gland were prepared in this manner. The final volume of solution injected amounted to about 2.0 cc. This solution was injected intra-abdominally into adult male white rats, bred from laboratory stock. The rats stood the injection well and showed no sign of distress immediately afterward or at any later time. The animals were kept in individual cages under laboratory conditions during the time necessary to complete the experiment. During this period they were given the usual food and water *ad libitum*.

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Mammalian thyroid extracts were prepared in the same manner, the necessary amount of commercial desiccated mammalian thyroid (tablet form) being dissolved in NaOH and neutralized with HCl. The synthetic thyroxine used in these experiments was prepared by Roche-Organon, one cc. of the solution containing 2 mgm. of thyroxine. Material for control injections was prepared by adding to the required amount of NaOH sufficient concentrated HCl to bring the pH of the solution to about 7.0.

Oxygen consumption was determined by placing the rat in a desiccator (volume 9500 cc.) through which air was forced under a slight positive pressure sufficient to give a flow of 500 to 670 cc. per minute. The flow was adjusted so that CO₂ concentration at equilibrium usually fell between 0.7 and 1.3 per cent. The animals quickly became accustomed to the apparatus and after briefly exploring their surroundings settled down and usually spent most of the time sleeping throughout the remainder of the test. Samples of air were taken after the rats had been in the chamber for two hours. They were analyzed with a Haldane-Henderson gas analyzer to determine the amount of oxygen which was removed and the amount of carbon dioxide which was added to the air during its passage through the chamber, the rate of flow at the time of sampling having been previously determined. It was then possible to calculate both oxygen consumption and CO₂ production during the test. The respiratory quotient and the oxygen concentration per hour per gram of body weight were then determined. The validity of the method is predicated, of course, on the assumption that no sudden change in oxygen consumption occurred during the test period, particularly within 10 minutes before collection of the sample.

The tests were run in the morning, after the rats had been left overnight in their cages with food and water. They were not fasted, therefore, prior to the test, since it is well known that rats under laboratory conditions usually feed at night and spend the daylight hours in sleep. Since repeated tests were desired on successive days extending over a period of a week or more, depriving the rats of food for this period would have produced such abnormal behavior as to invalidate the results. The relative constancy of the oxygen consumption of each rat over a period of several days prior to any experimental procedure indicated that the metabolic conditions prevailing during the tests were reasonably similar.

RESULTS

The purpose of the experiments was to determine the effect of parrot fish thyroid extract on the oxygen consumption, respiratory quotient and weight of adult male white rats and to make a comparison with the effects produced by synthetic thyroxine and desiccated mammalian thyroid powder.

Table I gives the results of 86 determinations of oxygen consumption and R.Q. in 22 non-treated rats. Their weights ranged from 250 to 500 grams (average 300 grams). Average oxygen consumption was 0.98 cc./gm./hr. and the average R.Q. was 0.84. As may be seen from an inspection of Table II, the values for the various test series before injection of thyroid preparations were in every instance close to these figures.

In Table II are shown the results of injecting various substances, known or suspected to contain active thyroid hormone, as compared to the effect of injecting

TABLE I

Oxygen consumption and respiratory quotient in non-treated white rats

Number of determinations	86
O ₂ consumption (cc./gm./hr.) Mean \pm SE _M	0.98 \pm 0.0129
Respiratory quotient Mean \pm SE _M	0.84 \pm 0.0070

TABLE II

Oxygen consumption and respiratory quotient in treated rats

Rat no.	Material injected	Before injection		Days after injection											
				1		2		3		4		7		10	
		R. Q.	O ₂ cons. cc./hr./gm.	R. Q.	O ₂ cons. cc./hr./gm.	R. Q.	O ₂ cons. cc./hr./gm.	R. Q.	O ₂ cons. cc./hr./gm.	R. Q.	O ₂ cons. cc./hr./gm.	R. Q.	O ₂ cons. cc./hr./gm.	R. Q.	O ₂ cons. cc./hr./gm.
8	Neut.	0.85	0.93	0.85	0.97	0.84	0.97								
4	NaOH	0.84	0.91	0.82	0.97	0.85	1.02								
1	2 cc.	0.82	1.08	0.77	0.93	0.81	1.03	0.84	0.93	0.85	0.80	0.74	0.92		
2A		0.83	1.12			0.76	0.99	0.79	0.91	0.79	0.95	0.84	0.89		
4A		0.78	1.12	0.81	1.01	0.79	1.08			0.81	1.12	0.84	1.01		
6A		0.84	0.98	0.76	1.01	0.81	0.89	0.80	0.91	0.81	0.95	0.79	0.96		
Ave.		0.83	1.02	0.80	0.98	0.81	1.00	0.81	0.92	0.82	0.96	0.80	0.95		
2	Thyroxine	0.76	1.02	0.79	1.34	0.69	1.44	0.72	1.16					0.93	0.99
7	1 mgm.	0.80	1.14	0.74	1.28	0.68	1.36	0.75	1.34					0.86	0.93
11	1 mgm.	0.85	0.91	0.79	1.22	0.69	1.39	0.74	1.15					0.81	0.90
0	0.2 mgm.	0.78	0.95	0.76	1.21	0.74	1.24	0.80	1.00						
13	0.2 mgm.	0.85	0.95	0.73	1.18	0.73	1.06	0.76	1.00						
Ave.		0.81	0.99	0.76	1.25	0.71	1.30	0.75	1.13					0.87	0.94
7A	Mammalian	0.83	0.90	0.69	1.13	0.74	1.08	0.72	0.97			0.94	1.03	0.95	0.88
8A	Thyroid 130 mgm.	0.84	1.06	0.80	1.31	0.82	1.25	0.75	1.36	0.78	1.28	0.76	1.45		
Ave.		0.84	0.98	0.75	1.22	0.78	1.16	0.74	1.17			0.85	1.24		
6	Fish	0.90	0.85	0.85	1.18	0.82	1.11	0.84	1.19			0.80	0.97	0.78	1.04
8	thyroid	0.85	0.93	0.80	0.99	0.87	0.92*								
	96 mgm.					0.78	1.23	0.73	1.19						
5	Av. dose	0.91	0.92			0.73	1.06	0.77	1.08						
Z		0.86	0.90	0.71	0.85	0.69	1.02	0.75	1.17	0.78	1.25	0.81	1.25		
K		0.93	1.01	0.77	1.16	0.74	1.38	0.78	1.17	0.75	1.20	0.84	1.10†		
3A		0.87	0.92	0.77	1.13	0.81	1.09	0.84	0.98	0.90	1.05	0.87	0.91		
5A		0.84	0.93	0.79	1.13	0.73	1.15	0.70	1.17	0.79	0.90	0.86	0.85		
Ave.		0.88	0.98	0.78	1.07	0.77	1.12	0.77	1.14	0.81	1.10	0.84	1.02		

* Re-injected with larger dose.

† Died on 8th day.

neutralized NaOH. These experiments are divided into four series, each of which is discussed separately below.

Neutralized NaOH solution. As a control on the handling and injection of the rats, neutralized NaOH solution was injected, in the same amount and manner as were the thyroid preparations. Six rats were so treated and in no instance was there any significant change in R.Q. up to 7 days after the injection. A slight fall in oxygen consumption during the experiment was probably attributable to adaptation.

Thyroxine. Synthetic thyroxine (Roche-Organon) was injected into 5 rats at two dose levels, 1.0 mgm. and 0.2 mgm. There appeared to be no appreciable difference between the effects of these two doses. Maximum effects on oxygen consumption and R.Q. were seen within two days after the injection.

Mammalian thyroid extracts. Commercial desiccated mammalian thyroid was extracted and injected in the manner described above. In two experiments 130 mgm. were given each time. There was a marked elevation of oxygen consumption, and a fall in R.Q. on the first day after the injection which continued until beyond the fourth day.

Fish thyroid extract. Extracts of parrot fish thyroid glands prepared in the manner already described were injected intra-abdominally into seven rats. The amount of dried gland extracted for this purpose varied between 69 and 110 mgm. In rat No. 8, 69 mgm. failed to produce an elevation in oxygen consumption or a fall in R.Q. by the second day after injection. Possibly this dosage was too small. On the second day after the original injection, therefore, the same animal was reinjected with 98 mgm. of dried extracted gland. Two days later it showed a marked rise in oxygen consumption and a fall in R.Q. In all other instances an elevation in oxygen consumption and a fall in R.Q. were observable within the first or second day after injection. In most cases oxygen consumption was still elevated on the seventh day, although the R.Q. usually had returned to the pre-injection level by that time.

Figure 1 shows graphically the average percentage changes in oxygen consumption and R.Q. All thyroid preparations produced increases in oxygen consumption and decreases in R.Q., while those rats injected with neutralized NaOH solution showed no percentage change in R.Q. and a perceptible decrease in oxygen consumption. This decrease is undoubtedly due to the adaptation of the animal to the respiratory chamber. As might be expected, the effect of synthetic thyroxine on oxygen consumption is more marked and of shorter duration than are the effects of extracts of fish and mammalian thyroid. Fish and mammalian thyroid injections increased oxygen consumption about 25 per cent at the maximum and the effects persisted for at least 10 days. There is a suggestion that fish thyroid has a somewhat more prolonged action than has mammalian thyroid. This also seems to be true of the effect on R.Q., which was still depressed 10 days after fish thyroid injection, but after mammalian thyroid injection it had returned to the pre-injection level within 7 days. The quantitative differences in the effects of synthetic thyroxine and extracted mammalian and fish thyroid are undoubtedly due to variations in the rate of absorption of the injected material.

No attempt has been made to determine the relative strengths of fish and mammalian thyroid. Since the amounts of the two extracts producing similar effects

were of the same order of magnitude, they are probably of the same approximate strength.

Figure 2 shows the effect of fish thyroid extract upon the weight of injected rats as compared to the effect of neutralized NaOH. In the four cases graphically por-

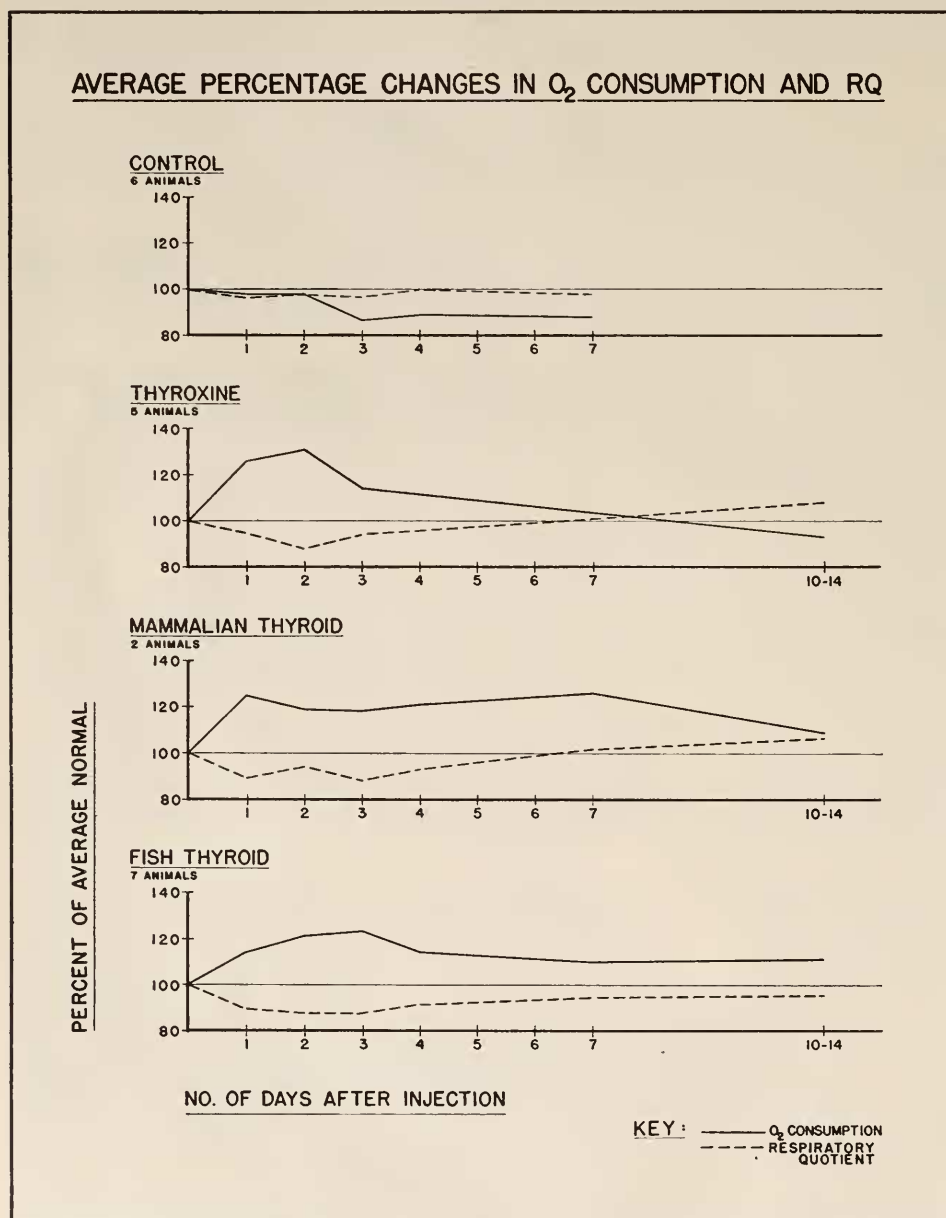


FIGURE 1. Showing the effect of thyroid extracts of fish and mammalian origin, as well as effect of thyroxine, on the oxygen consumption and respiratory quotient of white male rats.

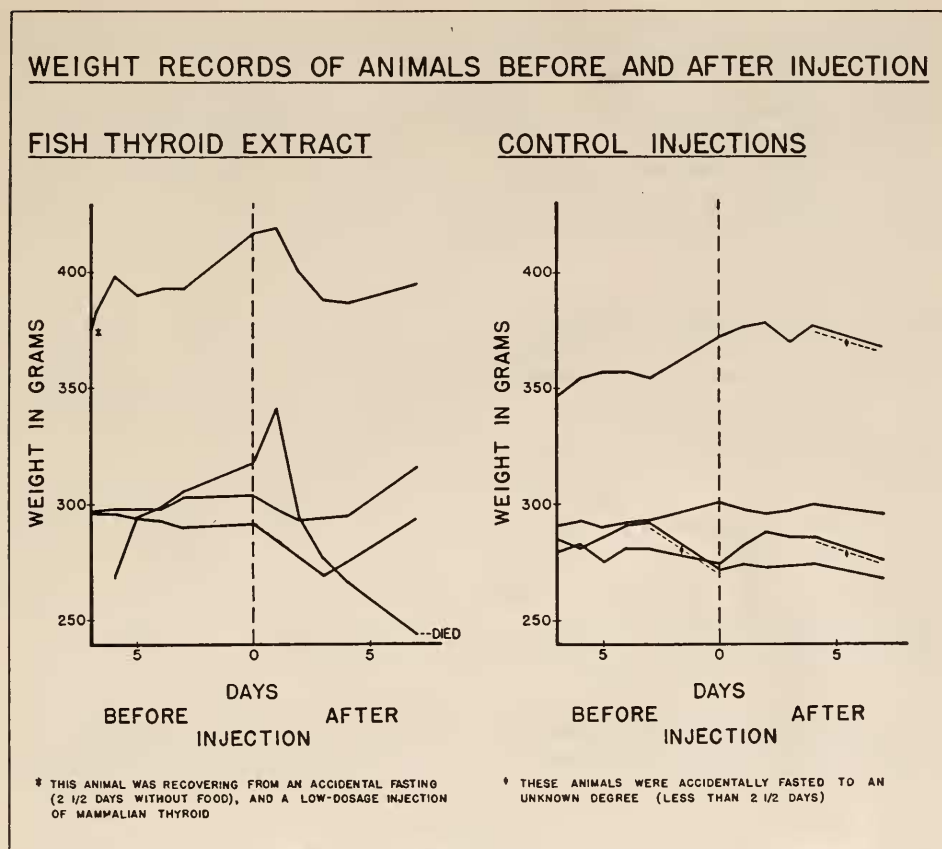


FIGURE 2. Showing effect of parrot fish thyroid extracts on the weight of white male rats.

trayed, fish thyroid extract invariably produced a marked fall in weight on the first or third day after injection, the fall persisting for at least 4 days. In most instances the weight was not recovered until at least one week after the injection. In those rats which were injected with NaOH solution, the weight of the animal following injection was, beyond the random variations, no different from the pre-injection value. While the effects of synthetic thyroxine and mammalian thyroid extract are not shown in this figure, their effects on weight are the same as that of the fish thyroid extract.

DISCUSSION

Data on the effect of thyroid extracts derived from the glands of lower vertebrates are virtually non-existent. The work done, however, is adequately considered in three excellent reviews on the comparative physiology of the thyroid which have appeared recently (Fleischmann, 1947; Goldsmith, 1949; and Lynn and Wachowski, 1951). These papers cover the subject so thoroughly that there is little point in reviewing the literature here except to summarize it briefly. Fleischmann (1947) notes only the effect of transplanted amphibian glands on amphibian

metamorphosis. There seems to be nothing else in the literature on the effect of amphibian extracts and nothing on the effect of reptilian extracts. Concerning the effects of fish thyroid extracts on fish, the only experiments reported are those of Smith and Matthews (1948) on the effect of parrot fish thyroid extracts on the oxygen consumption of white grunts. As to the chemistry of the thyroid hormones of lower vertebrates, we have only the data of Wolff and Chaikoff (1947) on shark and turtle thyroids, which give the thyroxine content in per cent of total iodine in sharks as 27.9 and in the turtle as 31.8, figures which are similar to those found in warm-blooded forms.

Sembrat (1927) reported acceleration of metamorphosis of tadpoles when implanted with bits of thyroid taken from either the dog-fish or the carp. Desiccated parrot fish thyroids (mixed with flour to form a paste) when fed to tadpoles will also produce premature metamorphosis according to Matthews and Ash (1951). Thyroid extracts prepared from glands taken from fish treated with propylthiouracil had no such effect. Thus, there is no doubt that the factor from the fish thyroid producing metamorphosis in amphibians is a true thyroid hormone.

While it is useless to speculate on the function of the thyroid in fishes because of the paucity of experimental data, it is not necessary to assume that it must serve a respiratory function associated with the regulation of body temperature. All attempts to increase the oxygen consumption of fish with mammalian thyroid extracts have failed (Drexler and Issekutz, 1935; Etkin, Root and Mofshin, 1940; Hasler and Meyer, 1942; Smith and Everett, 1943; and Matthews and Smith, 1947). However, extracts of parrot fish thyroid have been shown to elevate oxygen consumption in white grunts (Smith and Matthews, 1948). Since the increase was not consistently found in all injected grunts, there is some doubt as to the physiological significance of the elevation observed. It is not impossible that the respiratory stimulation is due to some sort of toxic reaction of the injected material. Nevertheless, the implication remains that teleost thyroid extract can elevate oxygen consumption and mammalian cannot. Matthews and Smith (1947) were unable to observe any change in oxygen consumption in *Fundulus* when injected with thiourea over a period of 5 to 6 days. It is possible, of course, that longer treatment with thiourea might have produced a different result. Further studies are necessary, including observations on the effect of fish thyroid extracts and thiourea on the R.Q. of fishes, before a final conclusion can be drawn.

There seems little doubt that the increase in respiratory metabolism observed in white rats after injection of thyroid extracts of the parrot fish thyroid is due to increased cellular oxidation. The exact parallelism between the effect of thyroid extract of mammalian origin as compared to that of fish origin leaves little room to question that the two are much alike in their physiological effects in the mammal. This view is strengthened by the fact that synthetic thyroxine behaves in the same way. The rise in oxygen consumption, fall in R.Q. and decrease in weight all follow the same pattern, regardless of the source of the thyroid hormone. This finding, coupled with the observations that (1) both desiccated fish thyroids and extracts of these glands will produce premature metamorphosis in amphibians, (2) the fish thyroid will concentrate iodine, and (3) chemically the thyroids of those lower vertebrates that have been studied (the shark and the turtle) are very similar to the

glands of higher vertebrates, makes it likely that the thyroid hormone of all vertebrates is much the same. It is, therefore, obvious that the teleost during the course of evolution has not sufficiently changed the nature of its thyroid hormone so that it may be distinguished from that of other vertebrates by ordinary physiological tests on amphibians and mammals. Whether the hormone still serves the same function in teleosts that it did in ancestral forms is at present unknown, just as it is not known whether the function of the hormone in mammals is a recent adaptation. The adaptation, if true, would appear to be on the part of the mammalian tissue to the hormone, rather than the other way around.

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SUMMARY

1. The effect of injecting extracts of parrot fish thyroid into adult, male white rats was to increase oxygen consumption, decrease respiratory quotient and decrease weight.
2. Injections of synthetic thyroxine or extracts of desiccated mammalian thyroid produced similar effects in respect to time of onset, intensity and duration of the responses.

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